Applicability of estimating glomerular filtration rate equations in pediatric patients: comparison with a measured glomerular filtration rate by iohexol clearance

FANG DENG, GAL FINER, SHANNON HAYMOND, ELLEN BROOKS, and CRAIG B. LANGMAN

CHICAGO, IL; AND HEFEI, CHINA

Estimating glomerular filtration rate (eGFR) has become popular in clinical medicine as an alternative to measured GFR (mGFR), but there are few studies comparing them in clinical practice. We determined mGFR by iohexol clearance in 81 consecutive children in routine practice and calculated eGFR from 14 standard equations using serum creatinine, cystatin C, and urea nitrogen that were collected at the time of the mGFR procedure. Nonparametric Wilcoxon test, Spearman correlation, Bland-Altman analysis, bias (median difference), and accuracy (P_{15}, P_{30}) were used to compare mGFR with eGFR. For the entire study group, the mGFR was 77.9 ± 38.8 mL/min/1.73 m^2. Eight of the 14 estimating equations demonstrated values without a significant difference from the mGFR value and demonstrated a lower bias in Bland-Altman analysis. Three of these 8 equations based on a combination of creatinine and cystatin C (Schwartz et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009;20:629–37; Schwartz et al. Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. Kidney Int 2012;82:445–53; Chehade et al. New combined serum creatinine and cystatin C quadratic formula for GFR assessment in children. Clin J Am Soc Nephrol 2014;9:54–63) had the highest accuracy with approximately 60% of P_{15} and 80% of P_{30}. In 10 patients with a single kidney, 7 with kidney transplant, and 11 additional children with short stature, values of the 3 equations had low bias and no significant difference when compared with mGFR. In conclusion, the 3 equations that used cystatin C, creatinine, and growth parameters performed in a superior manner over univariate equations based on either creatinine or cystatin C and also had good applicability in specific pediatric patients with single kidneys, those with a kidney transplant, and short stature. Thus, we suggest that eGFR calculations in pediatric clinical practice use only a multivariate equation. (Translational Research 2015;165:437–445)

Abbreviations: BUN = blood urea nitrogen; eGFR = estimated glomerular filtration rate; mGFR = measured glomerular filtration rate; Scr = serum creatinine; Scys = serum cystatin C

From the Division of Kidney Diseases, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, Ill; Division of Kidney Diseases, Department of Pediatrics, Ann and Robert H Lurie Children's Hospital of Chicago, Chicago, Ill; Department of Pediatrics, First Affiliated Hospital of Anhui Medical University, Hefei, Anhui Province, China; Department of Pathology and Laboratory Medicine, Ann and Robert H Lurie Children's Hospital of Chicago, Chicago, Ill; Department of Pathology and Laboratory Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Ill.

Submitted for publication July 3, 2014; revision submitted October 6, 2014; accepted for publication October 8, 2014.

Reprint requests: Craig B. Langman, Kidney Diseases, Ann and Robert H. Lurie Children's Hospital of Chicago, 225 E Chicago Avenue, #37 Chicago, IL 60611; e-mail: c-langman@northwestern.edu.

1931-5244

http://dx.doi.org/10.1016/j.trsl.2014.10.004

AT A GLANCE COMMENTARY

Deng F, et al.

Background

There are many estimating glomerular filtration rate (GFR) equations used in clinical practice. The bedside CKiD formula, based on creatinine only, is the most widely used formula in children. However, recent studies mainly in adults demonstrated that a combination of creatinine and cystatin C has superior performance. Few studies have evaluated estimating GFR equations in pediatric patients.

Translational Significance

This study translated the field of laboratory medicine for determining kidney function in children into an improved standard of clinical practice, by calculating the accuracy of multiple estimating equations through careful analysis of correlations' accuracy. When applied in 2 special populations, we found 3 equations to remain robust when compared with measured GFR.

INTRODUCTION

The glomerular filtration rate (GFR) is considered the best overall index of kidney function in health and disease. Thus, accurate measured GFR (mGFR) plays an important role in the clinical management of various diseases, both intrinsic to the kidney and with other diseases in which altered kidney function may influence the use of therapeutic agents, for example. More than 80% of clinical laboratories now report an estimating GFR (eGFR) when serum creatinine (Scr) is measured.[1] However, in recent years there are many studies that have shown that eGFR equations using additional markers of filtration, such as cystatin C, are superior to conventional equations based on Scr alone.[2,3] These equations were tested mainly in adult patients with chronic kidney disease (CKD), whereas only a few studies have evaluated performance of eGFR equations in pediatric CKD outside a research setting.

The most popular equation currently used in children is the 2009 Schwartz formula, which is based on Scr.[4] Despite standardization of Scr assays, eGFR remains relatively imprecise owing to variation in non-GFR determinants of Scr.[5] This equation does not differentiate between gender, despite the known gender difference in linear height and Scr concentrations, beginning in early adolescence. Thus, such anthropometric disparities result in a considerable variation in muscle mass and may be a dominant factor in eGFR differences.[6] Some studies in children have demonstrated that the inclusion of serum cystatin C (Scys) in the estimating equation increases the correlation with the mGFR than Scr alone.[7,8]

We compared 14 published eGFR equations against a gold standard mathematical model for mGFR from iohexol blood clearance[9] to guide clinicians in optimal eGFR determinations in a diverse group of children with possible kidney dysfunction. We hypothesized that the complex equation using gender, height, Scr, and Scys may be highly predictive of mGFR.

METHODS

Study design and data. This study was conducted at the Ann and Robert H. Lurie Children's Hospital of Chicago, Illinois (Lurie Children's), from November 2012 to January 2014. We used a single cross-sectional data set from 81 consecutive outpatients in which iohexol-based mGFR was calculated, based on the model used by Schwartz et al from the Chronic Kidney Disease in Children (CKiD) study,[9] and for which we are a participating center. At the time of the patient's mGFR study, additional data collected included Scr, Scys, blood urea nitrogen, visit date, anthropometrics, and demographics. We calculated height-for-age *Z*-score according to the United States Centers for Disease Control standards of recumbent length *Z*-scores, birth to 24 months, and stature *Z*-scores, 2–20 years in centimeters, by gender and age.[10] Fourteen eGFR equations were included and their respective values for 81 patients were compared against the mGFRs. This retrospective study was approved by the Lurie Children's Hospital of Chicago Institutional Review Board.

Laboratory analyses. We measured iohexol in serum by a validated liquid chromatography tandem mass spectroscopy method from 4 serial blood samples collected at 10, 30, 120, and 300 minutes postiohexol injection with the clearance calculated using the concentration of iohexol as a function of time in 2 curves (fast and slow plasma disappearance).[9] Scr was measured using an isotope-dilution mass spectrometry (IDMS)-traceable enzymatic method on the Roche Cobas 6000, following the Food and Drug Administration cleared procedure for Roche or Hitachi Cobas C systems. Blood urea nitrogen and cystatin C were analyzed in serum on the Roche Cobas 6000, following the Food and Drug Administration cleared procedures for Roche or Hitachi Cobas C systems. The cystatin C method on the Roche Cobas 6000 uses an automated particle-enhanced immunoturbidimetric assay (PETIA).

Table I. Published estimated glomerular filtration rate equations in children

Equation name	Equation
Scr based	
Schwartz et al[4] (ScrEq2009)	$41.3Ht/Scr$
Schwartz et al[11] (ScrEq2012)	$42.3(Ht/Scr)^{0.79}$
Gao et al[12]	$68(Ht/Scr) - 8(Ht/Scr)^2 + 0.48 \times age - 21.53$ in males or 25.68 in females
Pottel et al[13]	$107.3/(Scr/Q)$, $Q = 0.0270 \times age + 0.2329$
Hoste et al6	$107.3/(Scr/Q)$, $Q = 3.94 - 13.4\,L + 17.6\,L^2 - 9.84\,L^3 + 2.04\,L^4$
Scys based	
Bökenkamp et al[14]	$(162/Scys) - 30$
Grubb et al[15]	$84.69Scys^{-1.68} \times 1.384$ for age <14 years
Filler and Lepage[16]	$91.62(1/Scys)^{1.123}$
Schwartz et al[4] (ScysEq2009)	$41.9(1.8/Scys)^{0.777}$
Schwartz et al[11] (ScysEq2012)	$70.69Scys^{-0.931}$
Scr and Scys based	
Bouvet et al[17]	$63.2(1.2/Scys)^{0.56}\,(1.09/Scr)^{0.35}\,(weight/45)^{0.3}\,(age/14)^{0.4}$
Schwartz et al.[4] (ScrcysEq09)	$39.1\,(Ht/Scr)^{0.516}(1.8/Scys)^{0.294}(30/BUN)^{0.169}1.099^{male}(Ht/1.4)^{0.188}$
Schwartz et al[11] (ScrcysEq12)	$39.8\,(Ht/Scr)^{0.456}(1.8/Scys)^{0.418}(30/BUN)^{0.079}1.076^{male}(Ht/1.4)^{0.179}$
Chehade et al[18]	$42(Ht/Scr) - 4(Ht/Scr)^2 - 14.5Scys + 0.69age + 18.25$ for female or 21.88 for male

Abbreviations: *BUN*, blood urea nitrogen (in mg/dL); *Ht* or *L*, height (in m); *Scr*, serum creatinine (in mg/dL); *Scys*, serum cystatin C (in mg/L). Age is in years; weight is in kilograms.

eGFR calculation formulas. A total of 14 eGFR equations were selected to calculate eGFR (Table I). These include 5 equations based on Scr alone, 5 based on Scys alone, and 4 based on combinations of both. The method of testing Scys was particle-enhanced nephelometric immunoassay (PENIA) in Filler et al,[16] Bouvet et al,[17] Chehade et al,[18] and Schwartz et al[4,11] equations. The others used the PETIA method. The method of testing Scr was Jaffe method in Gao et al,[12] Bouvet et al,[17] and Chehade et al[18] equations. The others used the enzymatic assay.

Statistical analyses. Continuous data were described as the mean ± standard deviation, median, and interquartile range (IQR), and categorical variables were expressed as cases or percentages. Differences between eGFR and mGFR were analyzed by the nonparametric Wilcoxon test, because the data were not normally distributed. Correlations between eGFR and mGFR were established based on the Spearman correlation. Bland-Altman analysis was used to compare eGFR with mGFR using the average of the overall mean ± standard deviation and the precision was represented as the width between the 95% limits of agreement, wherein the smaller the limits of agreement, the greater the precision. Regression analysis and scatterplot analysis were used to compare the agreement between eGFR and mGFR. Three parameters used to assess the performance of eGFR equations relative to mGFR were as follows:

- Bias (median difference between mGFR and eGFR) and absolute bias (median difference in |mGFR − eGFR|;
- precision (IQR: P75-P25); and
- accuracy [percentage of estimates that differed within 15% of mGFR (P_{15}) and 30% of mGFR (P_{30})].

We selected $P < 0.05$ a priori to be statistically significant. Statistical analyses were completed using Statistic Package for Social Science (SPSS, Inc, Chicago, Illinois) and Medcalc (Medcalc Software, Mariekerke, Belgium).

RESULTS

Demographic and clinical characteristics. Characteristics of interest for our study population of 81 children and adolescents are shown in Table II. The minimum and maximum ages of the participants were 0.70 and 20 years, respectively. There were 10 patients with single kidney and 7 with a kidney transplant. The primary diseases that resulted in a kidney transplant were nephropathic cystinosis (4 cases), kidney dysplasia (2 cases), and autosomal recessive polycystic kidney disease (1 case). Five patients with Wilms tumor, 1 with mesoblastic nephroma, and 1 with Langer Giedion syndrome had single native kidneys after a unilateral nephrectomy performed for clinical care.

Analysis of the differences between the eGFR and mGFR values. The values of mGFR and the 14 corresponding eGFR values are shown in Table III. The mean mGFR for the 81 subjects was 77.9 ± 38.8 mL/min/1.73 m^2. The median and IQR (P_{25}, P_{75}) were 77.8, 52.0, and 96.0 mL/min/1.73 m^2, respectively. The numbers of patients with mGFR ≥90, 60–89, 30–59, and <30 mL/min/1.73 m^2 were 25, 31, 17, and 8,

Table II. Characteristics of study participants

Variable	Value
Measured glomerular filtration rate test, n	81
Age, y	
Mean ± standard deviation	12.60 ± 5.14
Median (P_{25}, P_{75})	14.29 (8.96, 16.88)
Gender, n (%)	
Female	37 (45.7)
Male	44 (54.3)
Ethnicity/race, n (%)	
White	47 (58.0)
Hispanic	22 (27.2)
Black	10 (12.3)
Asian	2 (2.5)
Weight (kg), median (P_{25}, P_{75})	46.30 (29.05, 60.40)
Height (cm)	
Median (P_{25}, P_{75})	152.30 (124.70, 167.60)
Z-score	−0.77 ± 1.97
Single kidney, n (%)	
Native kidney	3 (3.7)
After nephrectomy	7 (8.6)
2 Kidneys, n (%)	64 (79.0)
Kidney transplantation, n (%)	7 (8.6)
Primary kidney disease, n (%)	
Congenital anomalies of the kidney and urinary tract	16 (19.7)
Glomerular disease	6 (7.4)
Tubulointerstitial disease	5 (6.2)
Solid organ transplantation other than kidney	17 (21.0)
Metabolic disease	23 (28.4)
Other	14 (17.3)

Abbreviations: FSGS, Focal Segmental Glomerular Sclerosis; *p-ANCA,* perinuclear anti-neutrophil cytoplasmic antibody; *STEC-HUS,* shiga toxin escherichia coli hemolytic uremic syndrome. Glomerulopathies include 6 patients with microscopic polyangiitis, congenital nephrotic syndrome, thin basement membrane, FSGS, Kawasaki disease, and p-ANCA positive microscopic polyangiitis, respectively. Tubulointerstitial disease includes 5 patients with renal tubular acidosis, type I, Fanconi syndrome, interstitial nephritis, Bartter syndrome, and acute tubular necrosis, respectively. Solid organ transplantation other than kidney includes patients with a transplant of the liver (13), heart (1), lung (2), and bone marrow (1). Metabolic disease includes cystinosis (5), nephrolithiasis or hypercalciuria (12), and 6 patients with Hashimoto's thyroiditis, Lennox-Gastaut syndrome, tuberous sclerosis, Mainzer-Saldino syndrome, Langer Giedion syndrome, methylmalonic academia, respectively. "Other" are 5 patients with Wilms tumor, 3 with renovascular disease, 4 with mesoblastic nephroma, STEC-HUS and diabetes mellitus, autosomal recessive polycystic kidney disease, and neurofibromatosis, respectively, and 2 with unknown etiology of chronic kidney disease.

respectively. The calculated eGFR values were highly correlated ($P < 0.001$) with the mGFR value. However, 3 equations based on Scr alone, 1 based on Scys, and all 4 based on combinations of both demonstrated no significant difference from the mGFR values ($P > 0.05$). These same 8 equations also had lower bias compared with the others in the Bland-Altman analysis.

Consistency analysis of the eGFR and mGFR values. Table IV lists the performance of the selected 8 equations determined by calculating accuracy, bias, and precision. All had low bias, but 3 multivariate equations based on a combination of Scr and Scys, Schwartz et al[4,11] and Chehade et al[18] had the highest accuracy with approximately 60% of P_{15} and 80% of P_{30}.

Fig 1 shows the agreement between eGFR and mGFR for these 3 multivariate equations. There was good agreement across the GFR range from low to high, especially for equations of Schwartz et al.[4,11]

Analysis of the differences between the multivariate equation and mGFR in patients with single kidney, kidney transplant, or short stature. On the basis of the results mentioned previously, the 3 multivariate equations had the best performance among all eGFR equations. We analyzed their applicability in 10 patients with a single kidney, 7 with kidney transplant, and 11 short stature patients with height *Z*-score ≤ -2.5 (Table V). From the Wilcoxon test, there was no significant difference between eGFR and mGFR in patients with single kidney, kidney transplant, and short stature ($P \geq 0.05$). The values of the 3 equations also showed acceptable bias and precision in the Bland-Altman analysis.

DISCUSSION

Accurate assessment of GFR is essential for interpreting the symptoms, signs, and laboratory abnormalities that may indicate kidney disease, for monitoring side effects of therapeutic drug use, and for detecting and managing CKD and assessing its prognosis, among others. The gold standard for measuring GFR was inulin clearance for many years and was performed by loading and continuously infusing inulin and collecting timed urine samples from an indwelling bladder catheter, a procedure very cumbersome and difficult to perform in children.[19] Iohexol has been used as a satisfactory marker of GFR in adults and children, based on its ready availability, exclusive elimination by the kidneys without further metabolism, and good agreement with inulin and 51Cr-EDTA clearances. Indeed, iohexol has been heralded as the new gold standard measure of GFR and especially in children.[9,20]

In the present study, 8 of the 14 eGFR equations evaluated demonstrated better performance than the others compared with mGFR. These 8 were a mix of equations based on Scr only (3/5), Scys only (1/5), and a combination of both Scr and Scys (4/4). Further analysis demonstrated that only 3 specific multivariate equations had better performance than the univariate ones. These 3 equations all included Scr, Scys, gender, and a statural growth parameter. When used in unique patient

Table III. Overall limits of agreement between eGFR and mGFR

Equation name	Mean ± SD	Wilcoxon test		Correlation analysis		Bland-Altman analysis	
		Z	*P*	*r*	*P*	Bias	95% LOA
mGFR	77.9 ± 38.8	—	—	—	—	—	—
Schwartz et al[4] (ScrEq09)	*83.4 ± 48.5*	*−1.476*	*0.14*	*0.77*	*<0.001*	*−5.5 ± 26.0*	*−56.5; 45.5*
Schwartz et al[11] (ScrEq12)	71.2 ± 30.7	−2.229	0.03	0.77	<0.001	5.7 ± 20.0	−33.5; 44.9
Gao et al[12]	*76.4 ± 36.8*	*−1.490*	*0.14*	*0.73*	*<0.001*	*1.5 ± 50.0*	*−96.5; 99.5*
Pottel et al[13]	*85.1 ± 44.5*	*−1.923*	*0.054*	*0.72*	*<0.001*	*−7.2 ± 26.4*	*−58.9; 44.5*
Hoste et al[6]	84.9 ± 46.2	−2.281	0.02	0.78	<0.001	−7.0 ± 24.1	−54.2; 40.2
Bökenkamp et al[14]	126.5 ± 64.0	−7.573	0.00	0.81	<0.001	−48.7 ± 40.4	−127.9; 30.5
Grubb et al[15]	111.8 ± 81.3	−5.412	0.00	0.80	<0.001	−33.9 ± 54.6	−140.9; 73.1
Filler and Lepage[16]	94.1 ± 42.6	−5.605	0.00	0.84	<0.001	−16.3 ± 24.3	−63.9; 31.3
Schwartz et al[4] (ScysEq09)	65.9 ± 21.1	−3.962	0.00	0.84	<0.001	12.0 ± 24.6	−36.2; 60.2
Schwartz et al[11] (ScysEq12)	*71.2 ± 27.0*	*−1.937*	*0.053*	*0.84*	*<0.001*	*6.6 ± 22.5*	*−37.5; 50.7*
Bouvet et al[17]	*73.9 ± 31.1*	*−0.506*	*0.61*	*0.73*	*<0.001*	*3.9 ± 28.3*	*−51.6; 59.4*
Schwartz et al[4] (ScrcysEq09)	*77.9 ± 32.3*	*−0.445*	*0.66*	*0.87*	*<0.001*	*−0.0 ± 16.5*	*−32.3; 32.3*
Schwartz et al[11] (ScrcysEq12)	*76.2 ± 30.8*	*−0.186*	*0.85*	*0.88*	*<0.001*	*1.7 ± 16.6*	*−30.8; 34.2*
Chehade et al[18]	*74.4 ± 28.1*	*−0.308*	*0.76*	*0.79*	*<0.001*	*3.4 ± 31.2*	*−57.8; 64.6*

Abbreviations: eGFR, estimated glomerular filtration rate; *95% LOA*, 95% limits of agreement; *mGFR*, measured glomerular filtration rate; *r*, Spearman's correlation coefficient between eGFR and mGFR; *SD*, standard deviation; *Z*, value of the Wilcoxon test between eGFR and mGFR. The unit of GFR is mL/min/1.73 m^2. The italicized rows are equations for which there is no significant difference between eGFR and mGFR in the Wilcoxon test.

Table IV. Performance of the 8 equations in the overall sample

Equation name	Bias	Precision		Accuracy (%)	
	Median	IQR (P25, P75)	Absolute bias	P_{15}	P_{30}
Schwartz et al[4] (ScrEq09)	−3.1	27.1 (−16.6, 10.5)	14.0	39.5	65.4
Gao et al[12]	−2.9	25.4 (−16.7, 8.7)	11.5	51.9	71.6
Pottel et al[13]	−3.1	27.2 (−18.5, 8.7)	13.9	44.4	64.2
Schwartz et al[11] (ScysEq12)	1.9	19.6 (−5.0, 14.6)	8.6	53.1	79.0
Bouvet et al[17]	−0.2	30.1 (−14.0, 16.1)	15.2	34.6	64.2
Schwartz et al[4] (ScrcysEq09)	*−2.5*	*18.4 (−9.8, 8.6)*	*9.3*	*58.0*	*79.0*
Schwartz et al[11] (ScrcysEq12)	*−2.3*	*18.6 (−8.8, 9.8)*	*9.2*	*61.7*	*82.7*
Chehade et al[18]	*0.7*	*19.9 (−10.1, 9.8)*	*9.9*	*59.3*	*77.8*

Abbreviations: eGFR, estimated glomerular filtration rate; *IQR*, interquartile range; *mGFR*, measured glomerular filtration rate; *SD*, standard deviation.
Bias was the median difference between eGFR and mGFR (mGFR − eGFR); absolute bias was |(mGFR − eGFR)|; accuracy was calculated as the percentage of estimates of eGFR that differed from the mGFR within 15% (P_{15}) and within 30% (P_{30}). The italicized rows are the equations with the highest accuracy.

populations (ie, those with single kidney, kidney transplant, and short stature), the 3 equations demonstrated high agreement with mGFR.

There are only a few studies that have compared the applicability of eGFR equations based on different included variables in children. The performance of Scr-based equations was studied in several articles.[6,12,13] The bedside CKiD formula (Schwartz et al[4]) is the most widely used formula for eGFR in children. However it was derived from data obtained in children with CKD mGFR between 15 and 75 mL/min/1.73 m^2. Several recent studies validated new Scr-based formulas for children, which all outperformed the bedside CKiD formula compared with mGFR.[6,12,13] Sharma et al[21] studied several Scys-based equations and found the accuracy of various Scys equations varied with the actual mGFR. In a study focused on children with a solitary functioning kidney, the authors used 6 eGFR equations based on Scr, Scys, and a combination of both variables, and found the combined formula, Schwartz et al,[11] had superior precision.[22] For clinical practice, we need to identify the most accurate eGFR equation that can be applied to a diverse pediatric patient population. In adults, there are several large studies capable of validating the accuracy of eGFR equations. One recent example, the Chronic Kidney Disease Epidemiology Collaboration, developed an equation based on Scr in 2009 and 2 others

Table V. Agreements between multivariate equations and mGFR in special patients

Special patient	Mean ± SD	Wilcoxon test		Bland-Altman analysis	
		Z	*P*	Bias	95% LOA
Single kidney (n = 10)					
mGFR	66.5 ± 19.2				
Schwartz et al[4] (ScrcysEq09)	77.3 ± 22.0	−1.988	0.05	−10.8 ± 14.5	−39.2; 17.6
Schwartz et al[11] (ScrcysEq12)	75.0 ± 20.5	−1.682	0.09	−8.5 ± 13.6	−35.2; 52.2
Chehade et al[18]	77.5 ± 21.2	−1.988	0.05	−11.0 ± 15.3	−41.0; 19.0
Kidney transplant (n = 7)					
mGFR	63.0 ± 18.6				
Schwartz et al[4] (ScrcysEq09)	58.2 ± 18.1	−0.676	0.50	4.8 ± 12.5	−19.7; 29.3
Schwartz et al[11] (ScrcysEq12)	57.5 ± 17.5	−1.014	0.31	5.4 ± 12.1	−18.3; 29.1
Chehade et al[18]	57.9 ± 24.3	−0.845	0.40	5.0 ± 14.0	−22.4; 32.4
Z-score ≤−2.5 (n = 11)					
mGFR	59.7 ± 28.5				
Schwartz et al[4] (ScrcysEq09)	61.4 ± 20.8	−0.445	0.66	−1.7 ± 14.9	−30.9; 27.5
Schwartz et al[11] (ScrcysEq12)	59.8 ± 19.6	−0.445	0.66	−0.1 ± 14.7	−28.9; 28.7
Chehade et al[18]	64.7 ± 29.8	−0.978	0.33	−4.9 ± 19.0	−42.1; 32.3

Abbreviations: eGFR, estimated glomerular filtration rate; *95% LOA,* 95% limits of agreement; *mGFR,* measured glomerular filtration rate; *SD,* standard deviation; *Z,* value of the Wilcoxon test between eGFR and mGFR.
The unit of GFR is mL/min/1.73 m^2.

in 2012 (based on Scys alone and combined creatinine-cystatin C). They tested the 3 equations in very diverse populations with CKD and normal kidney function and found the combined creatinine-cystatin C equation performed better than equations based on either of both markers alone when compared with mGFR.[2] The combined equation is commonly used in adult hospitals as the method for eGFR in adults, replacing the popular Modification of Diet in Renal Disease eGFR.[3,23] Similarly to others in adults and children, we found that all 3 combined (Scr with Scys) equations outperformed equations that used the Scr or Scys alone.

Cystatin C is freely filtered and catabolized in the proximal tubules, without being secreted. Unlike Scr, it does not depend on gender or muscle mass and does not change with age between 1 and 50 years old.[24] Scys increases earlier than Scr as GFR decreases, so it may be a valuable marker in detecting early renal dysfunction.[25,26] In an early meta-analysis, Scys has also been reported to be superior to Scr for GFR estimation, particularly in patients with near-normal kidney function.[27] In addition to its use in estimating GFR, cystatin C has also been associated with subsequent adverse clinical events. In prior studies in the general population and in the elderly, cystatin C has been shown to be a better predictor of mortality and adverse cardiovascular events than Scr alone.[28-30] Peralta et al[31] studied cystatin C level in 11,909 participants and found its level may have a role in identifying individuals with CKD who have the highest risk for complications. The addition of cystatin C may improve mortality risk prediction by stages of kidney function relative to Scr.[32] In our study, all 3 combined equations with Scys exhibited superior agreement and performance, but each of those equations also included patient height and gender. However, including the height and gender does not explain totally the better performance of eGFR equations, because several other Scr-based equations used those variables as well. It is well known that a gender difference in the correlation of growth (height) and blood Scr concentration exists beginning in adolescence. This large variation in body shape and linear height determines extreme variations in muscle mass and may be a dominant factor when developing eGFR formulas for children, teens, and young adults.[6]

Higher cystatin C concentrations have been found in the first year of life previously. Bökenkamp et al[33] studied Scys level in 258 children without kidney disease, aged 1 day to 18 years, and found the cystatin C concentration was highest on the first days of life (range 1.64 ± 2.59 mg/L) with a rapid decrease during the first 4 months. Beyond the first year, the cystatin C concentration was constant. In a more recent study, Scys level was found to be a superior biomarker to Scr in the assessment of GFR in premature infants.[34] It is likely that the higher levels of cystatin C in the first year of life probably reflect the low GFR of neonates and infants. In our study, we only had 1 child under 1 year (0.7 years). There was a good agreement between mGFR and eGFR based on multivariate Schwartz equations.

It should be noted that creatinine and cystatin C methodologies differ among the various equations and systematic differences in measurement could contribute to the accuracy of the equations, given the methods

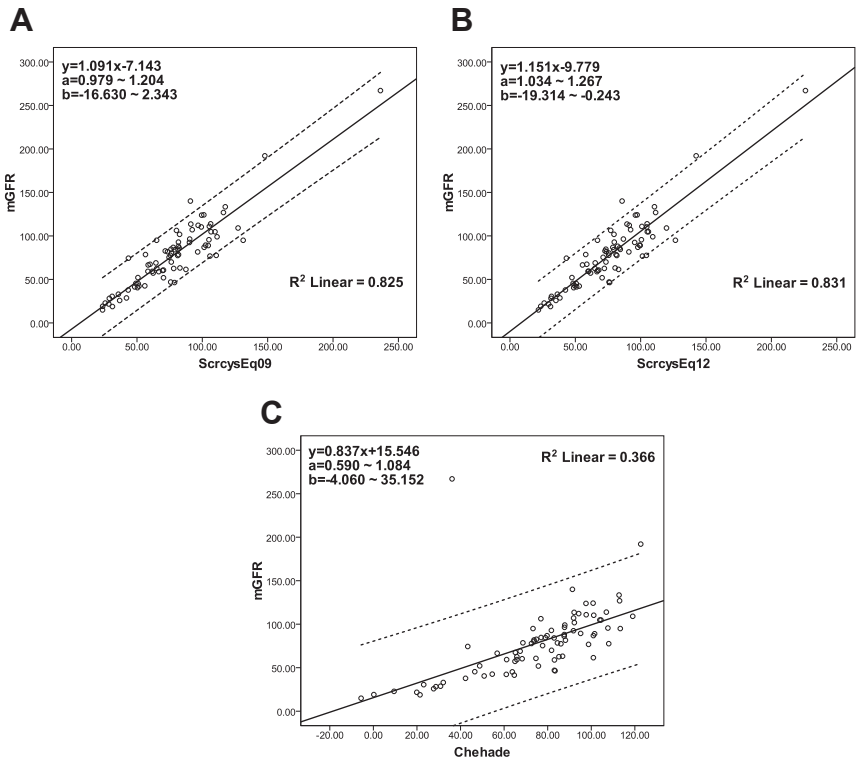

Fig 1. Scatterplot regression to analyze and compare eGFR with mGFR. **A**, Schwartz et al[4] (ScrcysEq09) eGFR equation explains 82.5% of the variability of mGFR. **B**, Schwartz et al[11] (ScrcysEq12) eGFR equation explains 83.1% of the variability of mGFR. **C**, Chehade et al[18] eGFR equation explains 36.6% of the variability of mGFR. [a]95% CI for the slope; [b]95% CI for the intercept; all $P < 0.001$. CI, confidence interval; eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate.

used in the present report. Because the relationship of both creatinine and cystatin C to GFR is exponential, the effect of analytical error (bias and precision) will be greater at lower or "normal" creatinine values (corresponding to high GFR) and the same difference will have minimal impact at highly abnormal creatinine values, which correspond to low GFR. Creatinine assays relying on both the Jaffe and enzymatic methods are now standardized to a material characterized by a gold standard method, IDMS-traceable method. Many of the equations evaluated herein used an enzymatic IDMS-traceable creatinine method, which is what we use at our institution. The Gao et al[12] Scr-only equation is based on a Jaffe IDMS-traceable method, and we found this equation, using our creatinine values, to have high agreement with mGFR.

The methodological differences noted between cystatin C assays lead to similar limitations that were historically experienced with creatinine and various eGFR equations. Efforts are now underway to calibrate different cystatin C methods to a single traceable reference material. The first report of a virtually assay-independent simple cystatin C-based eGFR equation based on calibration of different methods to an international reference material was recently published.[35] In the present study, our laboratory used a PETIA method on the Roche Cobas 6000 e501. Most of the equations evaluated reportedly used a PENIA method, most commonly that on the Siemens Bulk Nanocrystallized Ingot Iron platform. Hansson et al[36] showed in a comparison of 180 patient samples that Passing-Bablok regression analyses yielded a slope of 0.904 and intercept of 0.21 with regression coefficient of 0.9343 for cystatin C measured by Roche Cobas e501 cystatin C PETIA and Siemens Bulk Nanocrystallized Ingot Iron PENIA. Despite the limitations because of analytical differences among methods, we have shown that the combination of creatinine and cystatin C improves accuracy to mGFR.

The primary strength of this study is that it compares performance of 14 published eGFR equations in pediatric patients evaluated against an accurate and precise mGFR method in the routine clinical setting. The effects of different variables in the eGFR formulas were compared using a rigorous analytic plan to test the formulas against mGFR. Different analytic methods

demonstrated similar results for performance of each equation. No previous study has specifically assessed the comparison of these comprehensive equations in this age group. The limitations of this study include a relatively small sample of subjects, and the analysis was not based on CKD stage, owing to a relatively small number expected in some groups. However, in data shown from the scatterplot regression analyses, a stronger correlation can be seen with worsening CKD stage than in CKD stage 1, especially for the 2 Schwartz multivariate equations. Alternatively, the high overall correlation suggests that it would not have been different by differing stage of CKD with greater patient numbers within the lower bounds of mGFR.

CONCLUSIONS

The multivariate eGFR equations performed in a superior fashion than the univariate equations. The 3 eGFR formulas based on a combination of Scr, Scys, gender, and a growth parameter (Schwartz et al[4,11] and Chehade et al[18]) demonstrated exceptional accuracy among all formulas and had good applicability in special patients including those with a single kidney, kidney transplant, and short stature. Adding height and Scys to eGFR formula seems to be important in improving accuracy of the estimating equation. Our data suggest that for best accuracy to mGFR, all eGFR calculations in pediatric clinical practice use only multivariate equations, particularly 1 of the 3 mentioned previously. As this is a small study, our recommendations need to be confirmed in a larger sample size.

ACKNOWLEDGMENTS

Conflicts of Interest: All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

The study was supported in part by grants from the National Institutes of Health, HD 074596-02, DK666174, and DK083908-01 and by a grant, National Science Foundation of China, NSFC 81302447 from Dr Deng's hospital, First Affiliated Hospital of Anhui Medical University, Hefei, Anhui Province, China.

All authors have read the journal's authorship agreement. The manuscript has been reviewed and approved by all named authors.

REFERENCES

1. College of American Pathologists. Current status of reporting estimated glomerular filtration rate (eGFR). 2011 (http://www.cap.org/apps/cap.portal).
2. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012;367:20–9.
3. Lopes MB, Araújo LQ, Passos MT, et al. Estimation of glomerular filtration rate from serum creatinine and cystatin C in octogenarians and nonagenarians. BMC Nephrol 2013;14:265.
4. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009;20:629–37.
5. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.
6. Hoste L, Dubourg L, Selistre L, et al. A new equation to estimate the glomerular filtration rate in children, adolescents and young adults. Nephrol Dial Transplant 2014;29:1082–91.
7. Finney H, Newman DJ, Thakkar H, Fell JM, Price CP. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. Arch Dis Child 2000;82:71–5.
8. Dworkin LD. Serum cystatin C as a marker of glomerular filtration rate. Curr Opin Nephrol Hypertens 2001;10:551–3.
9. Schwartz GJ, Abraham AG, Furth SL, Warady BA, Muñoz A. Optimizing iohexol plasma disappearance curves to measure the glomerular filtration rate in children with chronic kidney disease. Kidney Int 2010;77:65–71.
10. Centers for Disease Control and Prevention. Growth charts Z-score data files. Available at: http://www.cdc.gov/growthcharts/zscore.htm.
11. Schwartz GJ, Schneider MF, Maier PS, et al. Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. Kidney Int 2012;82:445–53.
12. Gao A, Cachat F, Faouzi M, et al. Comparison of the glomerular filtration rate in children by the new revised Schwartz formula and a new generalized formula. Kidney Int 2013;83:524–30.
13. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. Pediatr Nephrol 2012;27:973–9.
14. Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C—a new marker of glomerular filtration rate in children independent of age and height. Pediatrics 1998;101:875–81.
15. Grubb A, Nyman U, Björk J, et al. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the modification of diet in renal disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. Clin Chem 2005;51:1420–31.
16. Filler G, Lepage N. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? Pediatr Nephrol 2003;18:981–5.
17. Bouvet Y, Bouissou F, Coulais Y, et al. GFR is better estimated by considering both serum cystatin C and creatinine levels. Pediatr Nephrol 2006;21:1299–306.
18. Chehade H, Cachat F, Jannot AS, et al. New combined serum creatinine and cystatin C quadratic formula for GFR assessment in children. Clin J Am Soc Nephrol 2014;9:54–63.
19. Arant BS Jr, Edelmann CM Jr, Spitzer A. The congruence of creatinine and inulin clearances in children: use of the Technicon AutoAnalyzer. J Pediatr 1972;81:559–61.
20. Schwartz GJ, Furth S, Cole SR, Warady B, Muñoz A. Glomerular filtration rate via plasma iohexol disappearance: pilot study for chronic kidney disease in children. Kidney Int 2006;69:2070–7.
21. Sharma AP, Yasin A, Garg AX, Filler G. Diagnostic accuracy of cystatin C-based eGFR equations at different GFR levels in children. Clin J Am Soc Nephrol 2011;6:1599–608.
22. Westland R, Abraham Y, Bökenkamp A, Stoffel-Wagner B, Schreuder MF, van Wijk JA. Precision of estimating equations

for GFR in children with a solitary functioning kidney: the KIMONO study. Clin J Am Soc Nephrol 2013;8:764–72.
23. Feng JF, Qiu L, Zhang L, et al. Multicenter study of creatinine- and/or cystatin C-based equations for estimation of glomerular filtration rates in Chinese patients with chronic kidney disease. PLoS One 2013;8:e57240.
24. Filler G, Bökenkamp A, Hofmann W, Le Bricon T, Martínez-Brú C, Grubb A. Cystatin C as a marker of GFR—history, indications, and future research. Clin Biochem 2005;38:1–8.
25. Sjöström P, Tidman M, Jones I. Determination of the production rate and non-renal clearance of cystatin C and estimation of the glomerular filtration rate from the serum concentration of cystatin C in humans. Scand J Clin Lab Invest 2005;65:111–24.
26. Massey D. Commentary: clinical diagnostic use of cystatin C. J Clin Lab Anal 2004;18:55–60.
27. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 2002;40:221–6.
28. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin-C and mortality in elderly persons with heart failure. J Am Coll Cardiol 2005;45:268–71.
29. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. N Engl J Med 2005;352:2049–60.
30. Astor BC, Levey AS, Stevens LA, Van Lente F, Selvin E, Coresh J. Method of glomerular filtration rate estimation affects prediction of mortality risk. J Am Soc Nephrol 2009;20:2214–22.
31. Peralta CA, Katz R, Sarnak MJ, et al. Cystatin C identifies chronic kidney disease patients at higher risk for complications. J Am Soc Nephrol 2011;22:147–55.
32. Driver TH, Scherzer R, Peralta CA, et al. Comparisons of creatinine and cystatin C for detection of kidney disease and prediction of all-cause mortality in HIV-infected women. AIDS 2013;27:2291–9.
33. Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Brodehl J. Reference values for cystatin C serum concentrations in children. Pediatr Nephrol 1998;12:125–9.
34. Abitbol CL, Seeherunvong W, Galarza MG, et al. Neonatal kidney size and function in preterm infants: what is a true estimate of glomerular filtration rate? J Pediatr 2014;164:1026–31.
35. Grubb A, Horio M, Hansson LO, et al. Generation of a new cystatin C-based estimating equation for glomerular filtration rate by use of 7 assays standardized to the international calibrator. Clin Chem 2014;60:974–86.
36. Hansson LO, Grubb A, Lidén A, et al. Performance evaluation of a turbidimetric cystatin C assay on different high-throughput platforms. Scand J Clin Lab Invest 2010;70:347–53.